Brain Penetrant CDK4/6 Inhibitor PRT3645 Demonstrates Anti-tumor Activity and Enhances Survival in Glioblastoma and Breast Cancer Brain Metastasis Models

Ashish Juvekar,[1] Yang Zhang,[2] Andrew Buesking,[1] Min Wang,[1] Dave Rominger,[2] Joseph Rager,[1] Stefan Ruepp,[1] Kirsten Gallagher,[1] Yue Zou,[1] Miles Cowart,[1] Xiaowei Wu,[1] Sarah Pawley,[1] Ryan Holmes,[1] William Gowen-MacDonald,[1] Kris Vaddi,[1] Andrew Combs,[1] Bruce Ruggeri,[1] Peggy Scherle[1]

[1]Prelude Therapeutics Incorporated, Wilmington, DE; [2]Quanta Therapeutics, Malvern, PA; contact: ajuvekar@preludetx.com

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Background

- Cell cycle deregulation is a hallmark of cancer and cyclin-dependent kinase (CDK) inhibitors specifically inhibit CDK4/6 and block cell transition from the G1 to the S phase of the cell cycle.[1]
- CDK4/6 inhibitors are the first and only class of highly specific CDK inhibitors approved for cancer treatment to date.[2,3]
- CDK4/6 inhibitors have transformed the treatment paradigm of estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 (HER2-) breast cancers, with three CDK4/6 inhibitors currently approved in the US.[2,3]
- Prelude Therapeutics, Inc. has discovered and developed PRT3645, an orally bioavailable brain-penetrant CDK4/6 inhibitor with high potency and selectivity, excellent pharmacokinetic (PK) parameters across species, brain penetrance, favorable tissue distribution relative to brain exposure, and significant anti-tumor efficacy in a subcutaneous model of breast cancer, orthotopic models of glioblastoma (GBM), and breast cancer brain metastasis (BCBM).

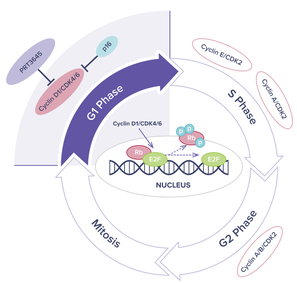
Objective

- To profile the biochemical/pharmacological activity of PRT3645, a brain-penetrant CDK4/6 inhibitor, both *In vitro* and *In vivo* in various cancers, including GBM and BCBM models, as a single agent as well as in combination with standard of care (SoC) agents.

Key Findings

- PRT3645 inhibits cellular phosphorylation of retinoblastoma (Rb) protein with low nanomolar activity.
- PRT3645 treatment resulted in inhibition of cell proliferation in various tumor types with most cell lines having a half-maximal inhibitory concentration (IC_{50}) of <100 nM.
- PRT3645 was well tolerated and highly efficacious in a subcutaneous xenograft model of breast cancer as well as in orthotopic models of human GBM and BCBM.

CyclinD-CDK4/6-Rb-E2F Pathway



Methods

- **Cell cycle analysis:** Cells were harvested, washed with phosphate-buffered saline (PBS), and fixed with ice-cold ethanol (70% v/v). Cells were stained following instructions from a Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit. DNA content was determined by DRAQ5™ DNA dye. Cell cycle distribution was analyzed using flow cytometry and the data were processed using FlowJo software.
- **Cell proliferation assay:** Cells were seeded in a 96- or 384-well plate, incubated overnight at 37 °C to allow adhesion, and then treated with inhibitors, as shown in the figures. Cell proliferation was determined using MTS solution (Promega) or fluorescence staining of nuclei.
- **Western blotting:** Briefly, cells were seeded at 5×10^5 cells/well in 6-well plates, incubated overnight at 37 °C to allow adhesion, and then treated with inhibitors for 24 hours. Cellular proteins were collected and denatured by 1% sodium dodecyl sulfate with β-mercaptoethanol.
- **Xenograft and orthotopic models (*In vivo*):** After tumors were established subcutaneously or orthotopically, treatment was commenced via oral gavage with either vehicle control or drugs, as described in the figures.

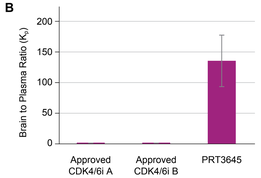
Results

Figure 1. PRT3645 Is a Potent and Selective CDK4/6 Inhibitor With High Brain Penetration Compared With Approved CDK4/6 Inhibitors

A

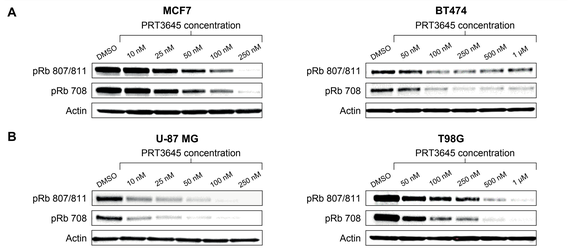
		PRT3645
Biochemical IC_{50} (CDK4, nM)[4]		3
Biochemical IC_{50} (CDK6, nM)[5]		14
Proliferation IC_{50} (MCF7, nM)		47
Proliferation IC_{50} (U-87 MG, nM)		21
Fold selectivity CDK4 vs other isoforms[5]	CDK1	>1000
	CDK2	>1000
	CDK3	>500
	CDK5	>1000
	CDK7	>1000
	CDK9	>1000

B



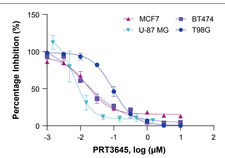
(A) Biochemical TR-FRET assay carried out at 1 mM ATP[5] or 2 mM ATP[4] and 10-day cell proliferation IC_{50} determined by CellTiter-Glo® assay. **(B)** Brain to plasma ratios (K_p) were determined following intravenous (IV) administration (bolus followed by 4-hour IV infusion) in male Sprague-Dawley rats (n=3). Bolus and infusion parameters were based on single bolus dose IV PK data and doses chosen to target a final steady state plasma concentration of 1 µM. ATP, adenosine triphosphate; TR-FRET, time-resolved Förster resonance energy transfer.

Figure 2. PRT3645 Inhibits Cellular Phosphorylation of Rb With Low Nanomolar Activity



Cells were treated in a concentration-dependent manner and western blotting analysis was performed for pRb 807/811 and pRb 708 in cells treated for 24 hours with PRT3645. Actin was used as a loading control. Proteins were visualized on the immunoblot by using LI-COR Odyssey® CLx system. **(A)** Breast cancer cell lines. **(B)** Glioblastoma cell lines. DMSO, dimethyl sulfoxide; pRb, phosphorylated retinoblastoma protein.

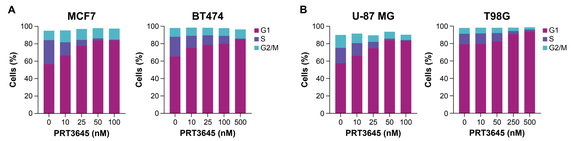
Figure 3. PRT3645 Inhibits Cellular Proliferation of Breast Cancer and Glioblastoma Cell Lines *In Vitro*



Cell Line	IC_{50} (nM)
MCF7	16
BT474	14
U-87 MG	7
T98G	94

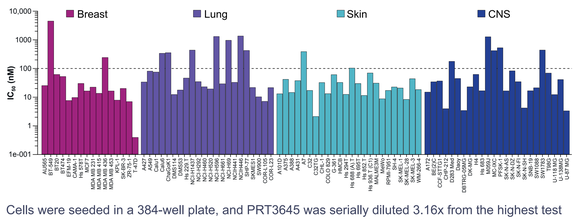
Breast cancer and glioblastoma cells were seeded in a 96-well plate, and PRT3645 was dispensed using a Tecan at ½log serial dilution. After a 10-day incubation, cell viability was measured using a cell counting kit-8 (CCK8) colorimetric assay that measures activity of dehydrogenases in cells which is directly proportional to the number of living cells. IC_{50} values were calculated by using GraphPad Prism 9.3.1 software.

Figure 4. PRT3645-Treated Cancer Cells Show Cell Cycle Inhibition With a Strong Reduction in the S-Phase



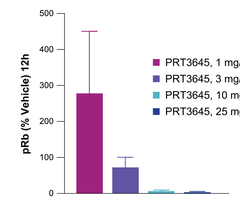
Cells were treated with PRT3645 in a concentration-dependent manner for 24 hours. Cell cycle phases (G1, S, and G2/M) were measured by EdU (5-ethynyl-2'-deoxyuridine) incorporation combined with DNA dye (DRAQ5™) for 2 hours. Samples were analyzed by flow cytometry, and the data were processed using FlowJo software. **(A)** Breast cancer cell lines. **(B)** Glioblastoma cell lines.

Figure 5. PRT3645 Is Highly Effective in Reducing Cell Viability of Various Tumor Types in an OncoPanel™ Cell Proliferation Assay



Cells were seeded in a 384-well plate, and PRT3645 was serially diluted 3.16x from the highest test concentration of 30 µM and assayed (OncoPanel™ cell proliferation assay) over 10 concentrations with a maximum assay concentration of 0.1% DMSO. Automated fluorescence microscopy was carried out using a Molecular Devices ImageXpress® Micro XL high-content imager, and images were analyzed with MetaXpress® 5.1.0.41 software.

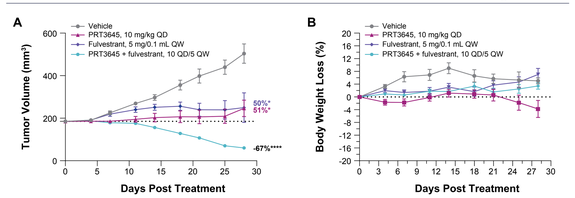
Figure 6. PRT3645 Is Highly Effective in Reducing Tumor pRb in a Single-Dose PK/PD Study in a U-87 MG Subcutaneous GBM Model



A U-87 MG subcutaneous GBM xenograft model was established by injecting tumor cells (3.5×10^5 cells/mouse, with 50% Matrigel™) in 0.1 mL media at the right flank by subcutaneous administration. PRT3645 was administered orally at 1, 3, 10, and 25 mg/kg as a single dose and tumor pRb levels were detected at the 12-hour timepoint.

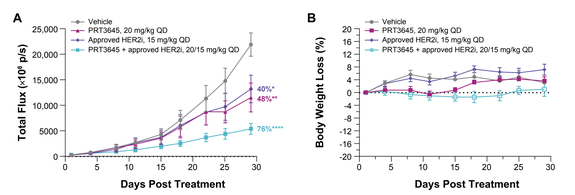
SD, single dose; PD, pharmacodynamics.

Figure 7. PRT3645 Is Highly Effective in Combination With Fulvestrant in a Subcutaneous ER+/HER2- MCF7 Breast Cancer Model and Shows Tumor Regressions



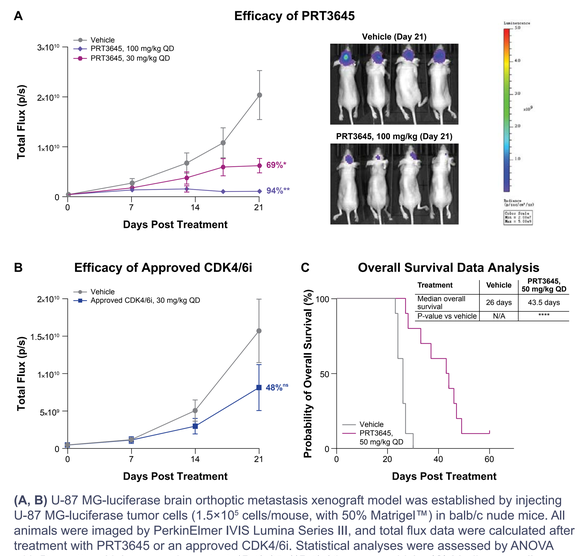
(A) Mice were inoculated subcutaneously at the right flank with MCF7 human breast tumor cells (10×10^6 cells) in 0.2 mL of PBS mixed with matrigel™ (1:1 v/w) for tumor development and 3 days before cell inoculation; β-estradiol sustained release tablets (0.36 mg) were implanted on the left back of each mouse. **(B)** Body weight changes post treatment. *P<0.05, ****P<0.0001 versus vehicle at day 28 post treatment. Statistical analyses were assessed by one-way analysis of variance (ANOVA) with Dunnett multiple comparisons test. QD, once daily; QW, once weekly.

Figure 8. PRT3645 Is Highly Effective in Combination With an Approved Small Molecule Brain Penetrant HER2 Inhibitor in ER-/HER2+ BT-474 Luciferase Orthotopic BCBM Model



(A) A BT-474 luciferase brain orthotopic metastasis xenograft model was established by implantation of 2.5 µL of cell suspension (1×10^5 cells/mouse with Matrigel™) into right caudate nucleus of female balb/c nude mice brain; tumors were measured with Xenogen IVIS® imaging and total flux data were calculated. **(B)** Body weight changes post inoculation. *P<0.05, **P<0.01, ****P<0.0001 versus vehicle at day 29 post treatment. Statistical analyses were assessed by ANOVA with Dunnett multiple comparisons test. HER2i, human epidermal growth factor receptor 2 inhibitor; QD, once daily.

Figure 9. PRT3645 Is Highly Efficacious and Enhances Survival in the U-87 MG-Luciferase Orthotopic GBM Model



Treatment	Vehicle	PRT3645, 50 mg/kg QD
Median overall survival	26 days	43.5 days
P-value vs vehicle	N/A	****

(A, B) U-87 MG-luciferase brain orthoptic metastasis xenograft model was established by injecting U-87 MG-luciferase tumor cells (1.5×10^5 cells/mouse, with 50% Matrigel™) in balb/c nude mice. All animals were imaged by PerkinElmer IVIS Lumina Series III, and total flux data were calculated after treatment with PRT3645 or an approved CDK4/6i. Statistical analyses were assessed by ANOVA with Dunnett multiple comparisons test; *P<0.05, **P<0.01 versus vehicle. **(C)** Median overall survival analysis and statistical analysis performed by using log-rank (Mantel-Cox test); ****P<0.0001 versus vehicle. ns, nonsignificant; QD, once daily.

Conclusions

- PRT3645 is an orally bioavailable, brain penetrant, and potent CDK4/6 inhibitor with >1000-fold selectivity against other CDK family members (CDK1, CDK2, and CDK9).
- Downregulation of pRb, reduction in S-phase of the cell cycle, and potent inhibition of cell proliferation in glioblastoma and breast cancer cell lines were observed with PRT3645 treatment *In vitro*.
- Across various tumor types, PRT3645 reduced cell viability with the majority of cell lines showing an IC_{50} value of <100 nM.
- PRT3645 single-dose administration in a subcutaneous U-87 MG GBM model showed dose-dependent reduction in tumor pRb at 12 hours.
- PRT3645 demonstrated significant efficacy in an ER+/HER2- MCF7 breast cancer xenograft model and resulted in tumor regression when combined with fulvestrant.
- PRT3645 was highly effective in reducing tumor burden in an ER-/HER2+ BT-474 luciferase orthotopic BC brain metastasis model and demonstrated significant combinatorial benefit with an approved small molecule brain-penetrant HER2 inhibitor in this model.
- PRT3645 was highly effective in reducing tumor burden in a U-87 MG-luciferase GBM orthotopic model and demonstrated enhanced median survival benefit.

References

1. Ettl T, et al. *Cancers (Basel)*. 2022;14:293.
2. Fasl A, et al. *Science*. 2022;375(6577):eabc1495.
3. Zhang M, et al. *Am J Cancer Res*. 2021;11:1913-1935.
4. Cozier L, et al. *EMBO J*. 2022;e108599.
5. Shagisultanova E, et al. *Mol Cancer Ther*. 2022;21:48-57.

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Disclosures

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